EDGEWOOD
RESEARCH, DEVELOPMENT & ENGINEERING CENTER
U.S. ARMY CHEMICAL AND BIOLOGICAL DEFENSE COMMAND

ERDEC-TR-425

PREDICTIVE BINDING PARAMETERS FOR DNA-DNA ASSOCIATION WITHIN A FLUID STREAM

Sheila J. Wood

RESEARCH AND TECHNOLOGY DIRECTORATE

July 1997

Aberdeen Proving Ground, MD 21010-5423

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

DEPARTMENT OF THE ARMY
U.S. Army Edgewood Research, Development and Engineering Center
Aberdeen Proving Ground, Maryland 21010-5423

ERRATUM SHEET

19 November 1997

REPORT NO.	ERDEC-TR-425
TITLE	PREDICTIVE BINDING PARAMETERS FOR DNA-DNA ASSOCIATION WITHIN A FLUID STREAM
AUTHORS	Sheila J. Wood
DATE	July 1997
CLASSIFICATION	UNCLASSIFIED

Please remove the front cover from copies of ERDEC-TR-425 sent to you earlier in 1997 and attach the enclosed replacement cover. Previously printed covers were inadvertently printed with the incorrect activity name and logo.

Sandra J. Johnson

SANDRA J. JOHNSON
Chief, Technical Releases Office

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave Blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	1997 July	Final, 93 Nov - 94 Nov

4. TITLE AND SUBTITLE
Predictive Binding Parameters for DNA-DNA Association Within a Fluid Stream

5. FUNDING NUMBERS
PR- 10161102BH67

6. AUTHOR(S)
Wood, Sheila J.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
DIR, ERDEC, ATTN: SCBRD-RTL, APG, MD 21010-5423

8. PERFORMING ORGANIZATION REPORT NUMBER
ERDEC-TR-425

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The ability to predict the rates of association of DNA to DNA have been used previously for those reactions occurring in a test tube. This study shows the ability to predict the binding rate of DNA to DNA within a fluid stream. The primary ligand was an oligonucleotide of 20 basepairs attached to the dextran matrix in BIAcore. Efficiency of hybridization of this ligand to a secondary ligand of 40 basepairs (containing 20 complementary) was assessed. Association was predictable, based on ssDNA remaining at equilibrium, using second order rate kinetics. Changes in concentration encompassing one order of magnitude had little to no effect on the efficiency of hybridization. Flow rates of 1 μL/min and 5 μL/min had no adverse effect on the efficiency of hybridization. All parametric observations encourage the use of DNA association within flow devices, and they emphasize the value of predictive indicators for establishing measuring times.

14. SUBJECT TERMS
DNA hybridization Affinity Binding rate kinetics

15. NUMBER OF PAGES
25

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

Blank

PREFACE

The work described in this report was authorized under Project No. 10161102BH67, Environmental, Pathogen Detection. This work was started in November 1993 and completed in November 1994.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release. Registered users should request additional copies from the Defense Technical Information Center; unregistered users should direct such requests to the National Technical Information Service.

Blank

CONTENTS

FIGURES

TABLES

Predictive Binding Parameters for DNA-DNA Association Within a Fluid Stream

1. Introduction

DNA hybridization studies have shown that predictions of association times and of hybridization efficiencies are possible. Given the starting and ending concentrations of single stranded (ssDNA), the G,C,A,T content of the DNA, the temperature, and the ionic conditions, hybridization times can be predicted as the reactions proceeds in a test tube. These parameters become important when predicting the importance of DNA hybridization as a tool for capturing and distinguishing microbes responsible for pathogenesis in humans. This study projects the use of DNA oligonucleotides to look at hybridization under very different conditions and uses conventional mathematical tools to predict binding rates.

Historically, hybridization rates were determined using a variety of analytical techniques. Radioligand techniques detect at picogram levels[1], and avidin-biotin couplings as signal molecules can detect at the nanogram level.[2,3] If the polymerase chain reaction (PCR) is used prior to signal generation, femtogram quantities equivalent to one genome can be detected.[4,5,6] Our approach measures hybridization as it occurs in real time at the nanogram level without the need for attachment of a signal generating molecule. Also, our approach circumvents the need to measure beginning and ending concentrations of ssDNA analytically.

In this study of hybridization rates, we used the unconventional environment of a real-time monitoring device, BIAcore ™ manufactured by Pharmacia Biosensor, Piscataway, New Jersey. The instrument measures molecular interactions as they occur. Biotinylated oligonucleotides were placed on a sensor chip and mass changes on the surface of the chip were monitored. Mass changes were a result of ligand binding at specific binding sites. The sensor surface was continually monitored for changes in the minimal angle of reflected monchromatic light. Mass changes sensitive and specific enough to calculate affinities of binding were measured. Changes in the minimal angle of reflectance were recorded as resonance units (RU) and converted to molar equivalents.

Although the ability to achieve DNA-DNA hybridization at room temperature, in five minutes under low stringency conditions (in BIAcore) has been shown previously[7], this study analyses the hybridization reaction using second order rate kinetic substitutions. Reactions followed to completion correlated with mathematical predictions of association rates. Minimal parametric changes had little to no effect on hybridization rates.

2. Materials and Methods

2.1 Source and description of materials

Oligonucleotides of 20 basepairs (bp) and 40 bp were obtained from Dr. Kim Rogers, USA Environmental Protection Agency, Las Vegas, Nevada. Avidin was obtained from Serva Biochemicals, Westbury, N.Y. Methods in BIAcore followed the original work as described by Wood. (7) A biotinylated 20 bp oligonucleotide (BUNI), a 40 bp (with 20 bp complementary to BUNI) oligonucleotide (BETA), and a 40 bp non-complementary oligonucleotide (FETA) were used.

2.2 Equilibrium experiments

Avidin was covalently immobilized to the sensor matrix using carboxyl amine coupling. The biotinylated oligonucleotide (BUNI) was injected followed by the 40 bp complementary strand (BETA). As BUNI and BETA hybridized, the reaction was followed for 10 minutes. The completed reaction was evaluated using second order rate kinetics.[8] Non-complementary FETA did not bind to BUNI in a separate experiment.

2.3 Kinetics experiments

Increasing concentrations of BETA were inoculated over a base of avidin/BUNI. Baseline was reestablished by washing with 100 mM HCl. Association rates for each concentration were obtained and the %BETA associated with BUNI was obtained using second order rate kinetics.

2.4 Parametric changes

Three differing concentrations of BETA at two differing flow rates were tested. The objective of this experiment was to observe any effects of minor parametric changes on hybridization.

3. Results

3.1 Equilibrium experiments

As shown in Figure 1, BUNI and BETA reached equilibrium at 300 seconds. Evaluation of the mathematical predictive value of second order rate kinetics suggests that single stranded DNA consumption can be predicted in BIAcore using this approach. (See Appendixes A, B, C, and D).

3.2 Kinetic evaluations

Figures 2 and 3 illustrate concentration dependent hybridization at a flow rate of 1 µl/min. Figures 4 and 5 illustrate the same concentration dependent hybridization using a flow rate of 5 µl/min. Conversion of RU to molar amounts at selected time points during the curve, allowed Cot curve substitution estimates of the relationship of bound to unbound single stranded DNA as the reaction progressed.

Concentration dependent reactions showed a 5-fold difference in RU response over a range of 1 uM to 9 µM BETA. Binding was linear at flow rates of 1 µl/min up to seven minutes for concentrations of 5 µM BETA or less. Non-linearity became apparent at concentrations of 7.5 and 9 µM BETA after nine minutes. At 5 µl/min, linearity was observed at concentrations of from 1 to 5 µM BETA Reactions slowed at concentrations of BETA above 5 µM. Association kinetics were measurable within a 5 minute time frame.

3.3 Parametric changes

As shown in Tables 1 and 2, changes in flow rate and concentrations did not affect the hybridization reaction between 5 and 9 minutes.

4. Conclusion

Our use of oligonucleotides in BIAcore resulted in the ability to predict rates of reaction based on mathematical assessment. Observations of concentration dependent binding and linearity of reactions provide the ability to decipher useful monitoring windows for a given set of reactions.

Figure 1. Resonance units increase as BUNI hybridizes to BETA, equilibrium at 300 seconds

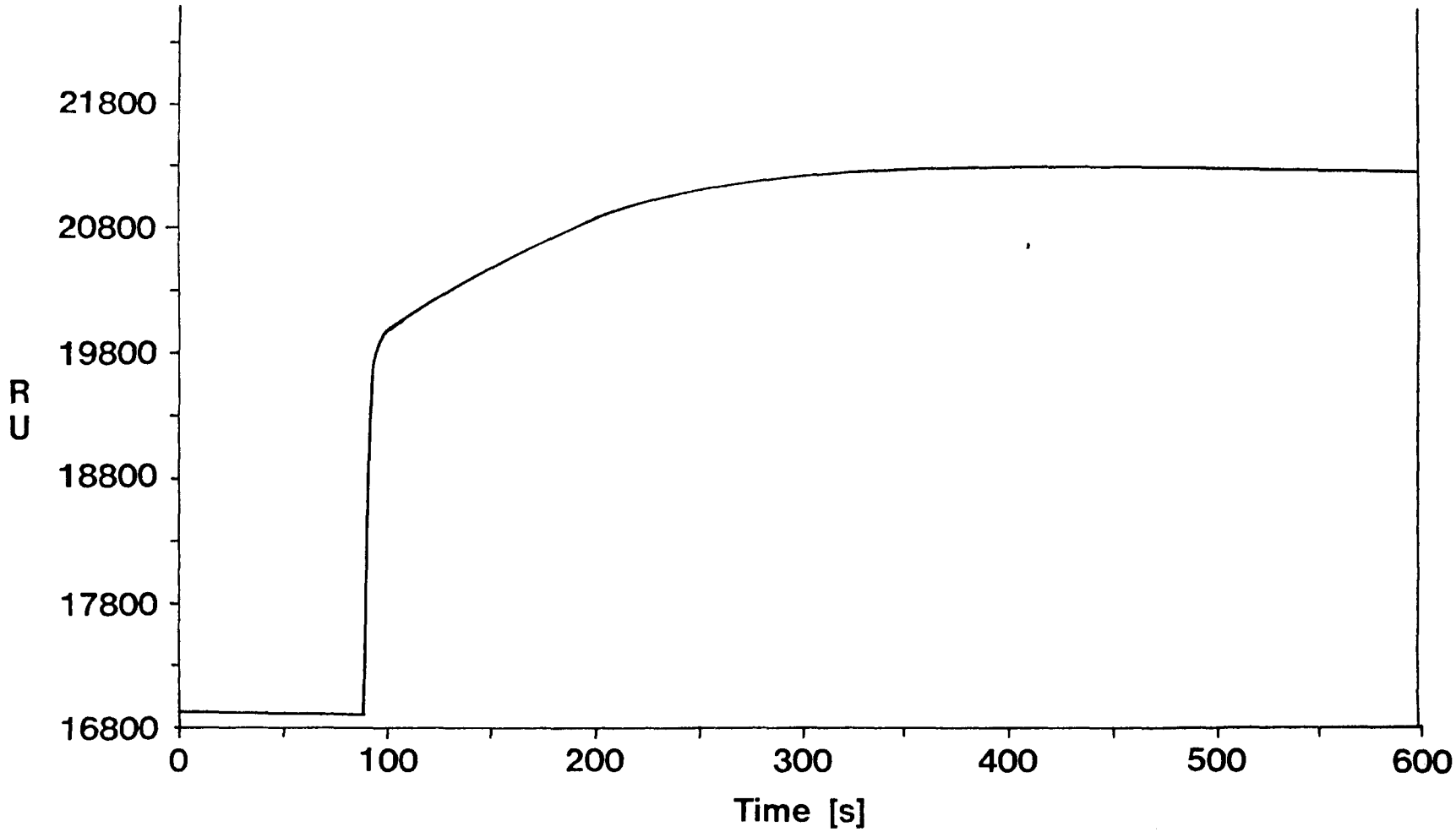

Figure 2. Percent BETA association at a flow rate of 1 µL/min

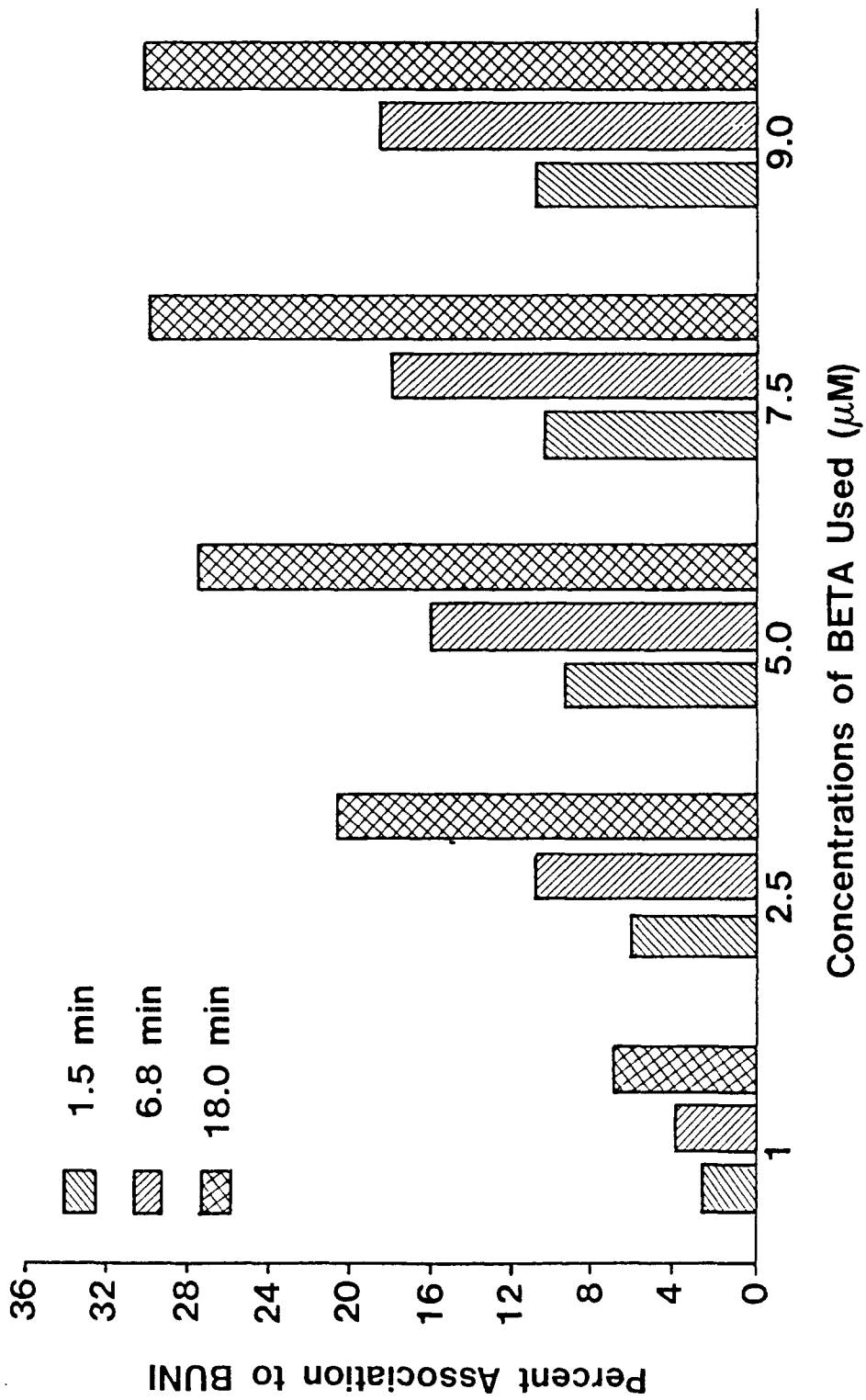

Figure 3. Relationship of concentration of reactants to percent association over time at a flow rate of 1 μL/min

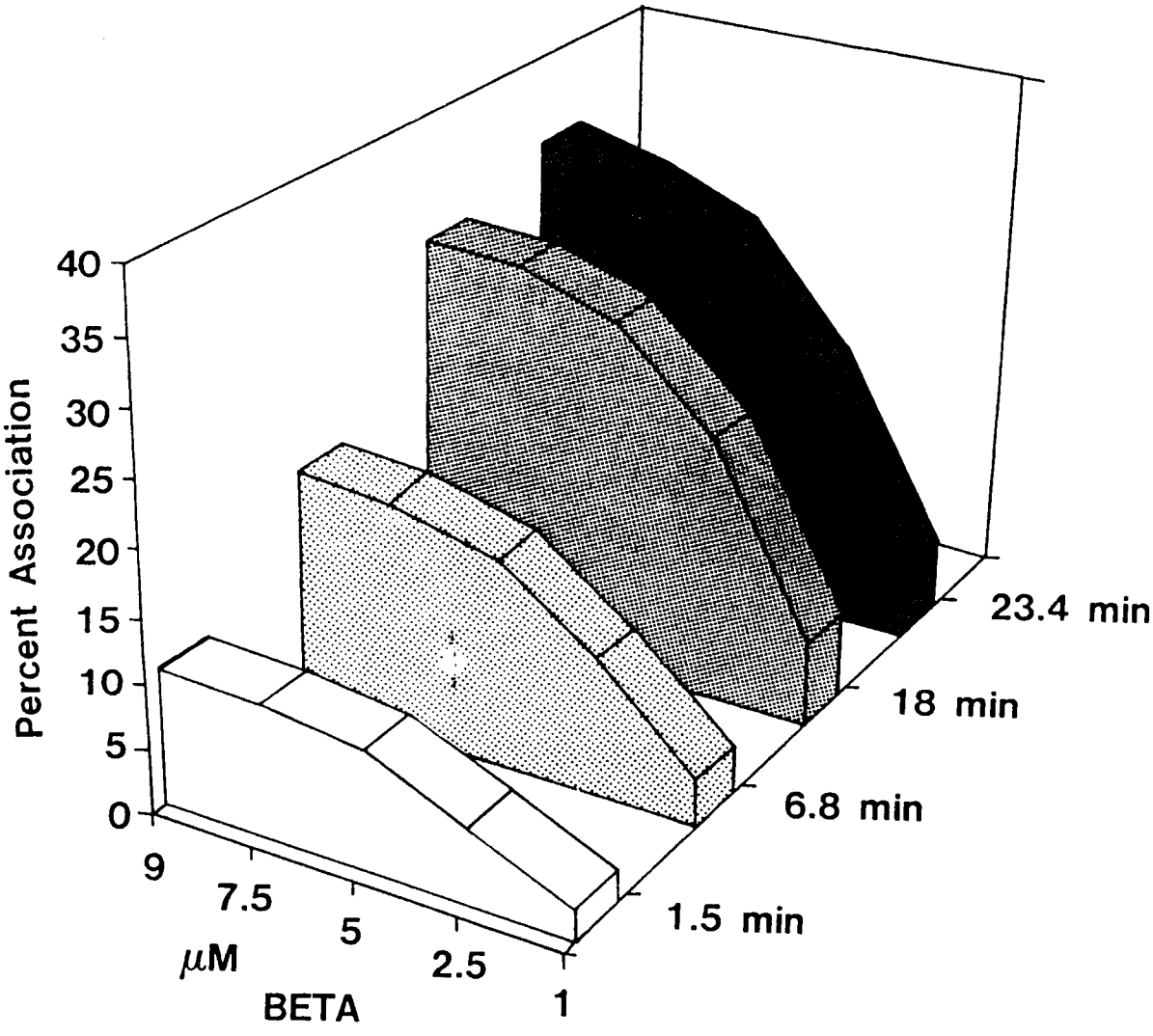

Figure 4. Percent BETA association at a flow rate of 5 µL/min

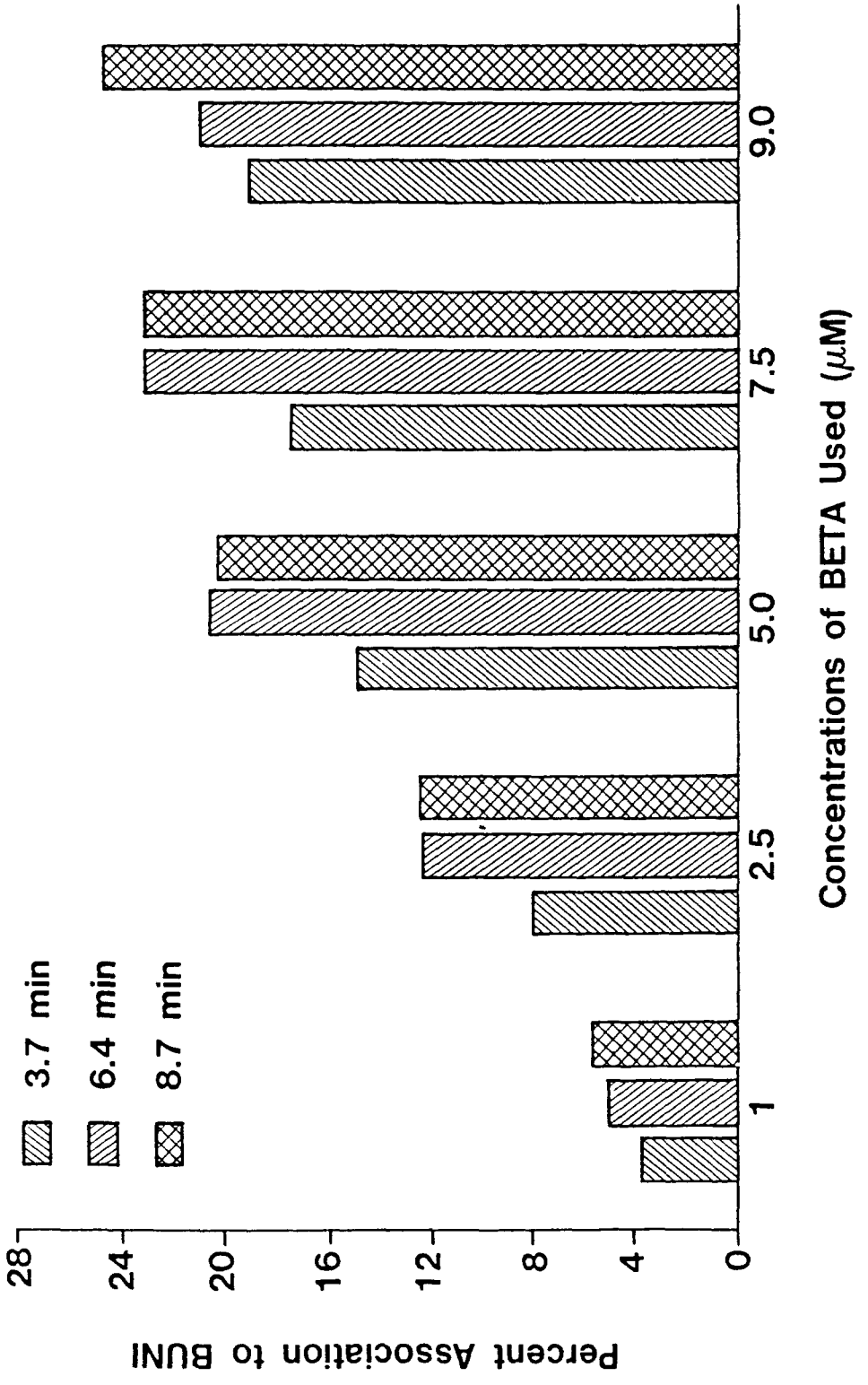

Figure 5. Relationship of reactant concentrations to percent association over time using a flow rate of 5 µL/min

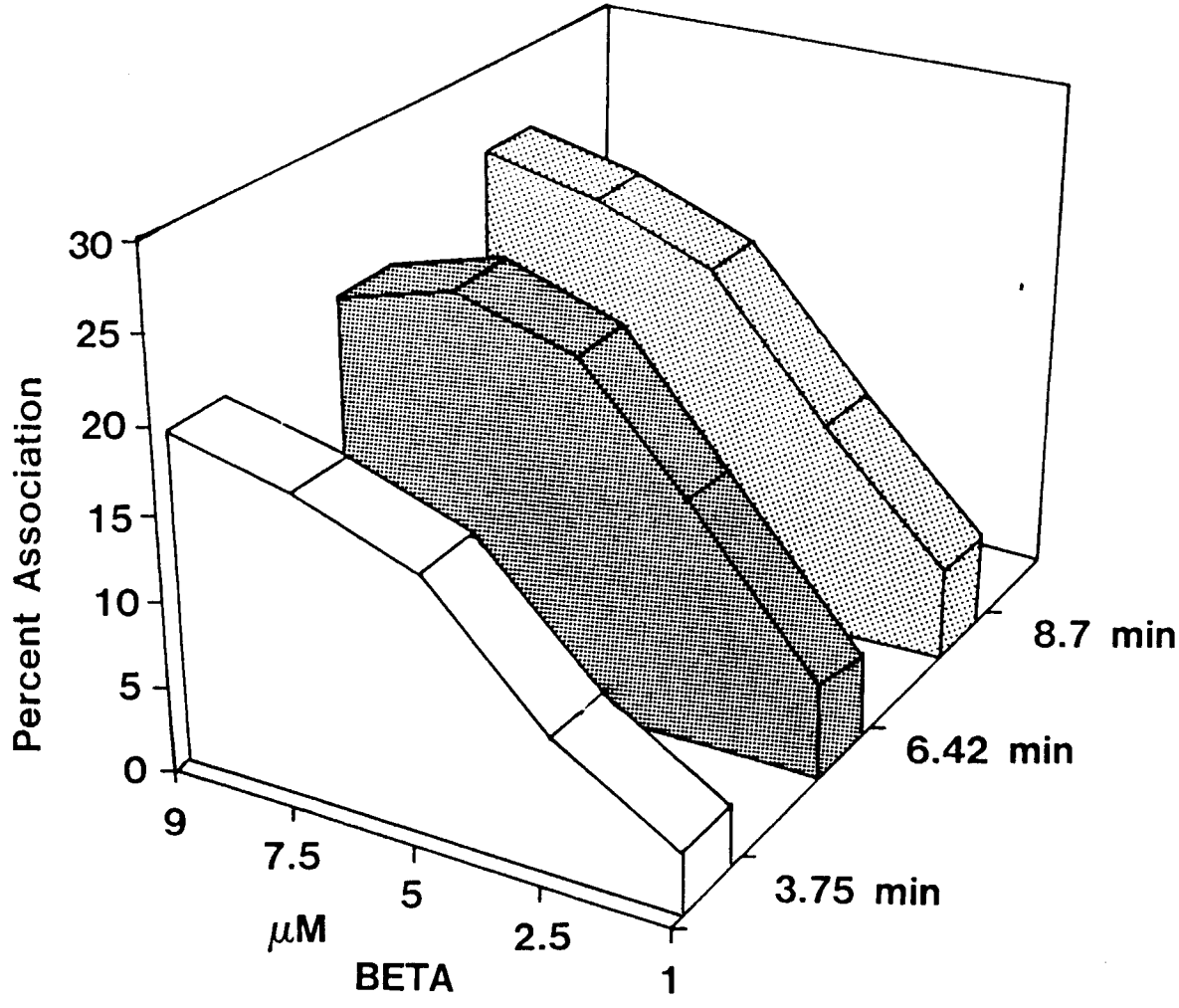

Table 1. Experimental Parameters Varying Flow Rate

Concentrations of Reactants Used in BIAcore at a Flow Rate of 1 μL/min

Avidin base	1.7 mM
BUNI attached	2.9 mM

BETA, 20 ul injections of the following concentrations:

- 9.0 μM
- 7.5 μM
- 5.0 μM
- 2.5 μM
- 1.0 μM

Calculations of the percent associated at the following times into the reaction:

- 1.5 min
- 6.8 min
- 18.0 min
- 23.4 min

Concentrations of Reactants Used in BIAcore at a Flow Rate of 5 μL/min

Avidin base	1.84 mM
BUNI attached	3.18 mM

BETA, 35 ul injections of the following concentrations:

- 9.0 μM
- 7.5 μM
- 5.0 μM
- 2.5 μM
- 1.0 μM

Calculations of the percent associated at the following times into the reaction:

- 3.7 min
- 6.4 min
- 8.7 min

Table 2. DNA-DNA association in response to minor changes in base probe concentration, measurement time, and flow rate

Ratio of Reacted to Unreacted DNA	Time	Base Conc BUNI	Conc BETA	Flow Rate
1/5.35	6.8 min	2.9 mM	9 μM	1 μl/min 20 μl
1/4	8.7 min	3.2 mM	9 μM	5 μl/min 35 μl
1/3.23	8.6 min	5.4 mM	9 μM	5 μl/min 35 μl

LITERATURE CITED

1. Martinez-Picado, J., Blanch, A.R., Jofre, J. Rapid detection and identification of *Vibrio anguillarum* by using a specific oliginucleotide probe complementary to 16s rRNA. Appl. Environ. Microbiol. 1994 60:732-737.

2. Leon, G., Maulen, N., Figueroa, J., Villanueva, J., Rodriquez, c., Vera, M.I., Krauskopf, M. A PCR based assay for the identification of the fish pathogen *Renibacterium salmoninarum.* FEMS. Microbiol. Lett. 1994 115: 131-136.

3. Haras, D., Amoros, J.P. PCR non-radioactive probes and clinical diagnosis. Diagnostic Clinique. 1994 4:43-52

4. Stonnet, V., Guesdon, J.L. *Campylobacter jejuni*: specific oligonucleotide and DNA probes for use in polymerase chain reaction-based diagnosis. FEMS Immunology and Medical Microbiology. 1993. 7:337-344.

5. Oyofo, B.A., Rollins, D.M.. Efficiency of filter types for detection of *Campylobacter jejuni* and *Campylobacter coli* in environmental water samples by polymerase chain reaction. Appl. Environ. Microbiol. 1993. 59:4090-4095.

6. Ho. M.S.Y., Conrad, P.A., Conrad, P.J., Lefevre, R.B., Perez, E., Bondurant, R.H. J. Clin Microbiol. 1994. 30:98-104.

7. Wood, S.J. DNA-DNA hybridization in real-time using BIAcore. Microchemical Journal. 1993. 47:330-337.

8. Johnson, J.L. Nucleic acid hybridization: principle and techniques. In: Nucleic Acid and Monoclonal Antibody Probes. Ed B Swaminathon, G. Prakash. Dekker, New York. 1989.

Blank

Appendix A BIAcore conversion formulas for proportionality of RU response to molar equivalents

Conversion formula for protein

$$\frac{\dfrac{\text{RU response}}{\text{x gm/L}} \times \dfrac{1000\ \text{RU}}{8.3}}{\text{ml wt of protein}} = \text{molar equivalent on matrix (sensor chip)}$$

Conversion formula for DNA

$$\frac{\dfrac{\text{RU response}}{\text{x gm/L}} \times \dfrac{1000\ \text{RU}}{8.3} \times (\text{x } 0.8)}{\text{ml wt of oligonucleotide}} = \text{molar equivalent oligo on matrix}$$

Blank

Appendix B Second order rate kinetics calculation for ssDNA

Co = ssDNA at time 0

C = ssDNA remaining at time t

k = equilibrium constant

C/Co = 1/[1 + (kCot)]

C/Co will equal 1/2 at equilibrium

therefore kCot = 1

Cot will equal 1/k

k will equal 1/Cot

Blank

Appendix C Experimental parameters for equilibrium experiments

Equilibrium Assessment Parameters; Flow Rate 5 μL/min

	Injection	Ml Wt	RU Response	Molar Equiv
Avidin	0.22U	66,000	6047	0.76 mM
BUNI	105 ng	7,000	1304	1.24 mM
BETA	150 ng	13,200	1312	0.66 mM

Blank

Appendix D Mathematical derivation of equilibrium conditions of DNA-DNA hybridizations in BIAcore

$C/Co = 1/(1 + kCot)$

$Co = 1240$ nM

$C =$ 580 nM at 300 seconds

$C/Co = 580/1240 = 0.47$

$kCot = 1$
$k(1240)(300) = 1$
$k = 2.6 \times 10\text{-}6$

$C/Co = 1/(1 + kCot)$

$0.47 = 1/ 1.9672$
$0.47 = 0.51$